Limiting Diversity of the CD8$^+$ T Cell Repertoire and T Cell Clonal Expansions: The Repercussions of Age on Immunity

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Abstract: The immune response diminishes with age. As a consequence, the elderly are highly susceptible to infection and exhibit an increased incidence of morbidity and mortality. One of the most profound changes observed in the aged immune system is the significant decline in diversity of the CD8$^+$ T cell repertoire, which occurs in both the naïve and memory T cell compartments. Understanding the mechanisms involved in the deterioration of the CD8$^+$ T cell repertoire is important for identifying strategies to improve immunity in the elderly. In this review we discuss the hypothesis that the naïve and memory CD8$^+$ T cell compartments become dysregulated by three distinct mechanisms. First, the naïve T cell repertoire progressively declines in diversity due to an age-associated decline in thymic output. Second, skewing of the naïve to memory T cell ratio in favor of memory T cells promotes activation of cross-reactive memory T cells that can mediate inappropriate responses. Third, the memory T cell repertoire is compromised by the appearance of T cell clonal expansions, which we propose are the natural outcome of long-term maintenance of the memory CD8$^+$ T cell pool that may occur independently of the ageing environment. Ultimately these events lead to a failure to mount effective immunity to both new and previously encountered pathogens.

Keywords: Ageing, CD8$^+$ memory T cell, influenza, repertoire diversity, T cell clonal expansion.

INTRODUCTION

A decline in immune responsiveness is one of the hallmarks of increasing age. As a result, the elderly are highly susceptible to infectious diseases such as influenza and respond poorly to vaccination [1-5]. Impaired immune function in the aged is associated with significant changes in the T cell repertoire, resulting from reduced thymic output [6-8], activation of cross-reactive memory T cells [9] and the appearance of large T cell clonal expansions (TCE)[10, 11]. In this review we will discuss how these three features of age and in particular the development of TCE, contribute to the diminishing diversity of the CD8$^+$ T cell repertoire, and how this impacts immune responses to new and previously encountered pathogens in aged individuals.

The naïve T cell repertoire is highly diverse, comprising millions of T cells with unique T cell receptor (TCR) molecules. In the aged, the TCR diversity is significantly reduced compared to young individuals [12, 13]. This is primarily a result of involution of the thymus that occurs with age and leads to a dramatic reduction in thymic output of T cells. In the absence of new thymic emigrants, the maintenance of naïve T cells in the periphery becomes increasingly dependent on homeostatic proliferation of the existing naïve T cell population [13, 14]. As a result, over time, the diversity of the naïve T cell repertoire declines. In addition, the decline in numbers of naïve T cells and increasing antigen experience leads to a dramatic skewing in the ratio of naïve to memory T cells in favor of memory T cells. It has been hypothesized that, cross-reactive memory T cells (originally generated in response to a different antigen) fill the hole in the T cell repertoire generated by the diminishing naive T cell pool and dominate the response to new pathogens [9]. Since cross-reactive memory responses may be of low affinity or mediate inappropriate responses, this may further compromise protective immunity [15-17].

Ageing also impacts memory CD8$^+$ T cell repertoire diversity through the appearance of TCE. These monoclonal expansions arise from a single CD8$^+$ T cell clone expressing a unique TCR and can occupy up to 90% (mouse) and 60% (human) of the CD8$^+$ T cell compartment [18, 19] resulting in reduction or depletion of other CD8$^+$ T cell clones in the CD8$^+$ T cell compartment. TCE are associated with chronic virus infections (e.g. cytomegalovirus), suggesting that chronic antigen stimulation is important for their development [20, 21]. Interestingly, increasing numbers of memory CD8$^+$ T cells has also been associated with chronic murine CMV infection, where CD8$^+$ memory T cells can represent up to 20% of the CD8$^+$ T cell population one-year post infection. These populations have been referred to as memory inflation. Importantly, this process could contribute to TCE development [22]. Mouse models have greatly contributed to our understanding of TCE biology and made it possible to determine the impact of TCE on immune responses to infections. Virus-specific TCE can develop within the memory T cell pool following acute respiratory virus infection [23, 24], and it has been proposed that TCE are a natural outcome of the long-term maintenance of the memory T cell pool. In addition, studies from mice demonstrate that TCE can also arise in specific pathogen free (SPF) animals in the apparent absence of chronic antigen stimulation [11]. Importantly, whether driven by chronic stimulation or not, the presence of

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a TCE severely compromises the overall diversity of the $CD8^+$ T cell repertoire, and can significantly impair the ability of the host to generate effective antiviral $CD8^+$ T cell responses to new and previously encountered antigens [24-26].

TCE are more frequently detected as individuals age, leading to the hypothesis that they are triggered by changes in the aged environment. However, it is possible that perturbations in the $CD8^+$ T cell pool occur early in life but are not readily detected by current techniques until they reach a certain size later in life. The gradual dysregulation of the T cell repertoire would ultimately result in the outgrowth of single $CD8^+$ T cell clones that dominate the antigen-specific T cell pool.

We propose that a combination of three separate, independent mechanisms contribute to the detrimental reduction in the size and diversity of the aged $CD8^+$ T cell repertoire, leading to impaired immune responses against both new and previously encountered pathogens: (i) thymic involution and the loss of the naïve T cell pool, (ii) skewing of the naïve to memory T cell ratio towards memory T cells and the activation of cross-reactive memory T cell responses and (iii) the gradual dysregulation of the memory T cell pool and the appearance of large TCE.

IMPACT OF AGEING ON THE NAÏVE T CELL REPERTOIRE AND THE ENSUING RESPONSE TO INFECTION

Age is a major complicating factor of many viral infections, including influenza. Approximately 90% of all influenza-related deaths occur in individuals greater than 65 years of age [27, 28], and the decline in immune function with advancing age is thought to be a major contributing factor.

The murine model is a valuable tool for the investigation of immune responses to influenza virus infection, and has provided useful insights to the role of individual components involved in protection against disease. It has been established that $CD8^+$ T cell responses play an important role in the clearance of established virus infection and can limit immunopathology associated with prolonged viral burden [29, 30]. Of importance, viral clearance is delayed in aged mice infected with influenza virus [31-33], which correlates with the slower kinetics of $CD8^+$ T cell responses to influenza compared to young mice [31-33].

In C57BL/6 mice, $CD8^+$ T cell responses are predominantly directed towards epitopes from internal viral proteins: nuclear protein ($NP_{366-374,}$ NP) and acid polymerase protein ($PA_{224-233,}$ PA) [34-37]. Repertoire studies indicate that T cells responding to NP exhibit a public repertoire, which consists of a limited TCR usage that is highly conserved among individual mice. In contrast, the T cells specific for PA exhibit a private repertoire, which is highly diverse and varies between individual mice [38]. In addition, the precursor frequency of T cells specific for NP in naïve young C57BL/6 mice is approximately 10 fold lower than the precursor frequency of T cells specific for PA [39]. Analysis of the diversity of anti-influenza $CD8^+$ T cell responses to these epitopes in aged mice revealed that the age-associated decline in the naïve T cell repertoire had a profound impact on responses to NP. Following influenza virus infection aged mice showed a dramatic reduction, and in some cases a complete loss of the NP-specific T cell response [40, 41]. Notably, the PA-specific T cell response in aged mice following influenza virus infection was similar to responses in young mice. The limited NP-specific response observed in the aged correlated with a decreased output of thymically derived naïve T cells, as young thymectomized mice also exhibit a reduction in NP-specific cells following *de novo* influenza virus infection. Furthermore, aged mice primed with the H3N2 mouse adapted HK-x31 influenza strain generated a significantly reduced NP-specific T cell response and impaired viral clearance following heterosubtypic H1N1 PR8 influenza infection [40]. Importantly, age-associated decline in T cell repertoire diversity had a significant impact on control of infection, but did not correlate with impaired function of the T cell clones in the aged [42-44]. These data suggest that contractions in the size and diversity of the naïve $CD8^+$ T cell repertoire caused by ageing can result in a preferential decline in responses with low naïve T cell precursor frequency, ultimately leading to holes in the repertoire. In summary, the age-associated decline in repertoire diversity and loss of reactivity to immunodominantepitopes correlate with both impaired cellular immunity to *de novo* influenza virus infection and a compromised recall response to heterosubtypic infection.

The Impact of Cross-reactive Memory T Cells on Immune Responses in the Aged

Despite the loss of naïve T cell repertoire diversity, it has been shown that pre-existing memory T cells that dominate the $CD8^+$ T cell pool in the aged have the potential to cross-react with newly encountered, unrelated antigen [15-17, 45]. Therefore cross-reactive memory T cells may make an important contribution to the response to newly encountered antigens in mice as the naïve T cell repertoire becomes increasingly limited with age [9]. Of note, cross-reactive memory T cells can have a profound impact on the characteristics of the ensuing response in terms of T cell specificity and immunopathology, which may alter the ability to control infection [46-48]. For example, cross-reactive responses involve a restricted repertoire of T cells, which are most likely of low avidity and limited to a few epitopes, which could be directed toward subdominant epitopes that initiate overzealous responses to non-pathogenic antigens. In summary, the age-associated decline in naïve T cell size and repertoire diversity not only impairs immune responses to newly encountered pathogens, but can also drive responses to new antigens by cross-reactive memory T cells, resulting in inappropriate and possibly detrimental responses to infection.

DEVELOPMENT OF T CELL CLONAL EXPANSIONS FURTHER LIMITS THE DIVERSITY OF THE T CELL REPERTOIRE

Increasing age has been associated with the appearance of TCE. TCE can comprise a significant fraction of the total T cell pool and impact the diversity of the $CD8^+$ T cell repertoire and subsequent immune responses to new and previously encountered pathogens. In both humans and mice there appears to be two classes of TCE based upon their independ-

ence or dependence on chronic antigen stimulation. Currently, it is not known what role antigen experience has on the development of these different TCE subsets.

Characteristics of TCE of Unknown Specificity

TCE can be identified on the basis of TCR variable α (Vα) and variable β (Vβ) usage. Typically 60% of aged inbred mice housed in SPF environments show extensive perturbations in the frequencies of the different TCR Vα and Vβ gene segments. For example, aged mice bearing TCE have a marked increase in the representation of a particular TCR-Vβ element within the $CD8^+$ T cell pool that steadily increases over time and varies between individuals [11]. In contrast, young mice exhibit a remarkably conserved distribution of different TCR Vα and Vβ genes in the $CD8^+$ T cell population.

Phenotypic analysis of TCE expressing a particular TCR-Vβ element, isolated from SPF mice revealed they express markers commonly associated with resting memory T cells, such as CD44, CD127, CD122 and do not express markers associated with recent antigen activation, such as CD25 and CD69. Moreover, TCE are phenotypically indistinguishable from non-TCE memory cells within the same individual [49, 50]. Interestingly, Clambey *et al* identified two distinct populations of TCE based on the expression of the integrin receptor CD49d, which facilitates lymphocyte tissue homing. $CD49d^+$ TCE were characterized by decreased expression of the homeostatic cytokine receptors, CD127 (IL-7Rα) and CD122 (1L-2Rβ), an impaired ability to proliferate in response to mitogenic stimulation *in vitro*, and were not detected in mice greater than 18 months of age. In addition, $CD49d^+$ TCE displayed markers associated with exhaustion (PD-1) and terminal differentiation (KLRG-1). In contrast, $CD49d^-$ TCE were more commonly found in older SPF mice and persisted over the lifetime of the individual. $CD49d^-$ TCE proliferated in response to mitogenic stimulation, expressed CD127, CD122 and were not functionally exhausted [51]. Of note, $CD44^+$ $CD49d^-$ $CD8^+$ T cells that are specific for model antigens were recently identified in young unimmunized SPF and germ free mice [52]. These memory-like T cells exhibit enhanced homeostatic proliferation and cytokine production compared to their $CD8^+$ $CD44^-$ counterparts. This study suggests that T cells of the same specificity have distinct fates, which are generated as a result of homeostatic proliferation. It is possible that the varying disposition to undergo homeostatic proliferation by distinct subsets of $CD8^+$ T cells in young mice plays a potential role in the gradual generation of TCE in the aged.

Antigen-specific TCE arise from the Memory T Cell Pool

One major question in the field is the origin of TCE of unknown specificity. One likely possibility is that they are memory cells from past antigen encounters. Consistent with this, TCE from SPF mice commonly express CD44, a marker of antigen experience, possibly indicating specificity for environment antigens.

Recently it was formally demonstrated that antigen-specific TCE could indeed arise from the memory T cell pool. This was first observed in a cohort of Sendai (murine parainfluenza virus) and influenza virus infected mice that were bled monthly to determine the frequency of antigen-specific $CD8^+$ T cells in the circulating T cell pool [23]. Large clonal outgrowths (sometimes representing 90% of the total $CD8^+$ T cell pool) expressing a single Vβ were detected within the virus-specific memory T cell pool as early as 12 months post infection. Antigen-specific TCE express cell surface molecules consistent with resting memory ($CD44^{hi}$ $CD25^{lo}$ $CD69^{lo}$) and are phenotypically indistinguishable from non-expanded antigen-specific memory $CD8^+$ T cells. When restimulated with peptide *in vitro*, TCE produced comparable levels of both IFNγ and TNFα to young memory $CD8^+$ T cells [23, 24], in agreement with the observations from other studies examining the properties of TCE [49-51, 53]. Thus, these studies directly demonstrate that antigen-specific TCE can develop from the memory T cell pool following acute virus infection.

Impact of TCE on Immune Responses to New and Previously Encountered Pathogens

Our understanding of how TCE impact immune responses to infections stems from work using TCE with unknown specificity [25, 26]. These studies show that the presence of a TCE significantly limits the availability of T cells capable of responding to new infections, resulting in increased susceptibility to infection. Recent work by our lab investigated whether Sendai-specific TCE could mediate recall responses to a secondary Sendai respiratory virus infection. A dual adoptive transfer approach was used to directly compare two memory T cell populations isolated from two congenically distinct mice strains transferred into a third congenic recipient (Fig. **1**). Immune responses of the two memory populations were then tracked and measured in the same recipient under the same infection conditions. Memory $CD8^+$ T cells from seven individual mice bearing large TCE were compared to memory $CD8^+$ T cells from young Sendai infected mice. Memory T cells isolated from five out of the seven mice that exhibited TCE expanded ten-fold less than memory $CD8^+$ T cells from young Sendai infected mice ([24], unpublished data). Of note, we isolated the total $CD8^+$ memory T cell pool from TCE bearing mice and therefore non-TCE Sendai-specific memory T cells were also included in the donor population. To tease apart the responses of the TCE and non-TCE memory cells within the population, Vβ expression was determined before and after *in vivo* recall. Cells expressing the dominant Vβ at the time of transfer did not expand, indicating a failure of the TCE to respond to antigen stimulation ([24], unpublished data). These studies suggest that virus-specific TCE not only limit the repertoire of cells capable of responding to infection but also exhibit impaired recall responses to their specific (or cognate) antigens. In summary,TCE have a negative impact on immune responses to new and previously encountered pathogens as demonstratedfrom studies analyzing TCE of unknown specificity [25, 26] and virus-specific TCE [24] respectively.

CD49d and CD62L Expression: Can they Predict Origin and Function of Antigen-specific TCE?

Interestingly, although the experiments described above indicate that most TCE are poorly responsive, large TCE from two mice that were identified mounted recall responses

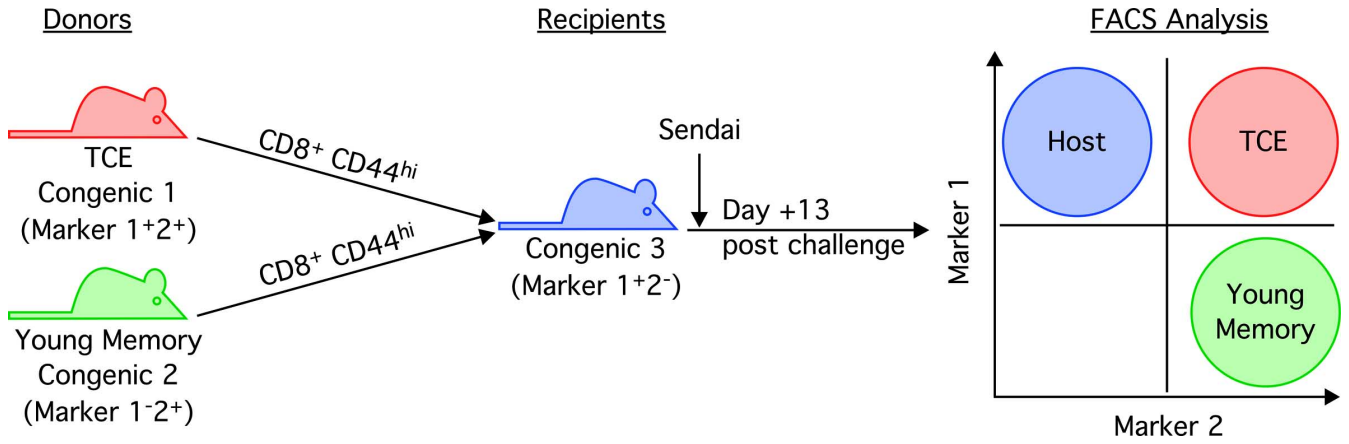

Fig. (1). Measuring the recall response to secondary virus infection by antigen-specific TCE. Memory $CD8^+$ T cells ($CD44^{hi}CD8^+$) were isolated and sorted from a mouse (congenic 1) bearing a TCE (Sendai-specific $CD8^+$ T cells > 20% of $CD8^+$ T cell pool) and from mice (congenic 2) infected with Sendai virus 1 month earlier (young memory). TCE and young memory $CD8^+$ T cells were then mixed and transferred into naïve recipients (congenic 3) at equal numbers of Sendai-specific cells. Recipient mice were infected i.n. with Sendai virus 1 day following transfer. At the peak of the immune response, flow cytometry analysis was performed to determine the frequency of Sendai-specific $CD8^+$ T cells from each congenically distinct population. The recall response of TCE was determined as a ratio of young memory Sendai-specific $CD8^+$ T cells to TCE Sendai-specific $CD8^+$ T cells.

equivalent to or greater than young memory $CD8^+$ T cells. This demonstrated that not all TCE are functionally alike ([24], unpublished data). The differential recall efficiencies of TCE may reflect different TCE subsets that have been previously described [51]. However, differences in the ability of TCE to mount recall responses failed to correlate significantly with CD49d expression (Fig. **2a**).

Phenotypic analysis of T cell subsets can be useful for determining the function or origin of a particular T cell. Interestingly, CD62L expression on TCE isolated from mice and humans differ. TCE from humans express little to no CD62L [54], whereas TCE isolated from individual Sendai-infected donors expressed varying levels of CD62L (Fig. **2b**). Notably, TCE that exhibited equivalent or greater recall responses than young memory $CD8^+$ T cells following secondary challenge expressed high levels of CD62L (Fig. **2b**).

Memory T cells are not uniform with respect to function, proliferation or trafficking and can be loosely categorized into one of two broadly defined subsets: central memory T cells (Tcm) or effector memory T cells (Tem) [55]. Tcm cells express CD62L and CCR7 and primarily reside in secondary lymphoid organs. Upon antigen exposure, Tcm rapidly divide but do not produce effector cytokines immediately after activation. Conversely, Tem lack the expression of CD62L and CCR7, are predominantly found in peripheral tissues, and can mount rapid recall responses producing effector cytokines after secondary contact with antigen [56]. Notably, the relative contribution of Tcm and Tem to the protective recall response is not well understood. One possibility is that Tem, which accumulate in peripheral sites mediate early, short-lived responses to secondary pathogenic encounters. By contrast, Tcm residing in lymphoid organs may initiate a delayed but sustained immune response, maintaining the memory T cell pool. It is not clear whether TCE that fall into the Tem or Tcm subset mediate different recall responses. Of note, not all TCE expressing CD62L mounted effective recall responses following secondary Sendai virus infection (Fig. **2b**). Thus, further investigation is required to determine the role of CD62L expression on TCE during recall responses. Currently it is unclear whether cell surface receptor expression can provide a useful indicator to the function and origin of virus-specific TCE.

DO HOMEOSTATIC CYTOKINES CONTRIBUTE TO THE DEVELOPMENT AND MAINTENANCE OF T CELL CLONAL EXPANSIONS?

The outgrowth of a single $CD8^+$ T cell clone does not generally affect the overall size of the total $CD8^+$ T cell compartment; instead other $CD8^+$ T cell clones decrease in size or are completely depleted from the population [10]. Determining the mechanisms that allow a $CD8^+$ T cell clone to outcompete other clones within the $CD8^+$ T cell pool is critical to understanding how memory immune responses are maintained in the elderly and what factors control TCE development. It has become increasingly clear that TCE maintain a higher rate of cell division compared to non-TCE memory T cell counterparts, contributing to their selective survival [24, 49, 50]. Adoptive transfer of either TCE from SPF mice or non-TCE cells from age-matched controls into naïve mice showed that TCE undergo more cell division compared to non-TCE cells, as determined by CFSE dilution assays [49]. Interestingly, in some recipients, transferred TCE populations began to dominate the recipient T cell pool over time [50]. Furthermore, in a cohort of Sendai infected mice treated with BrdU for 14 days beginning 600 days post infection, Sendai-specific $CD8^+$ TCE demonstrated a preferential uptake of BrdU [24]. In agreement with this study, Ku *et al* have also shown this to be true for TCE isolated from SPF animals [49]. Thus, the increased rate of homeostatic turn over by individual $CD8^+$ T cell clones contributes significantly to the development of these expansions.

Although the data indicate that TCE turn over more rapidly than non-TCE, regulation of their homeostasis is poorly understood. In the case of normal T cells, the cytokines IL-7

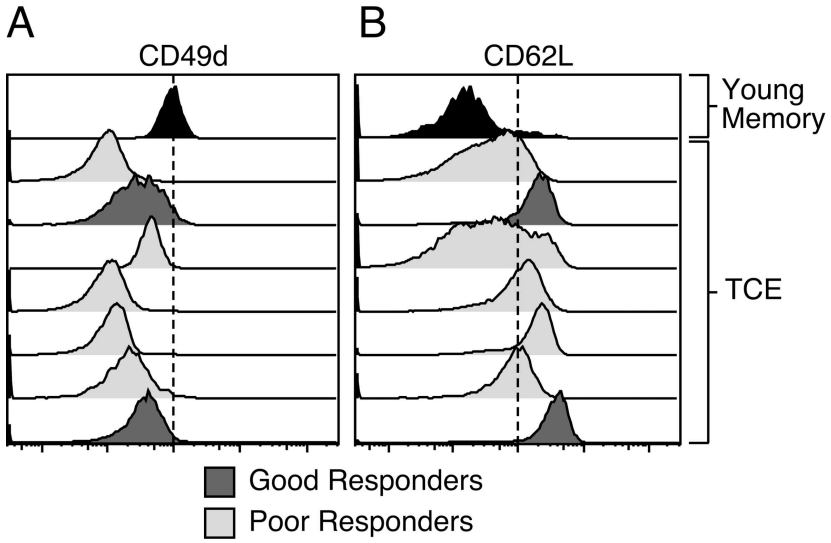

Fig. (2). CD49d and CD62L expression by antigen-specific T cell clonal expansions. Antigen-specific TCE isolated from 7 Sendai infected mice >300 days post infection. CD49d (**a**) and CD62L (**b**) expression by TCE was determined by flow cytometry.

and IL-15 have been shown to play an essential role in their maintenance and survival. Mice that lack the ability to produce or respond to these cytokines fail to generate or maintain memory T cell populations [57-59]. Like normal memory T cells, optimal proliferation by TCE is dependent on IL-15. In the absence of IL-15 signaling, TCE proliferation is substantially reduced [49]. However, TCE division is not completely prevented, and other factors may be important for their homeostasis. Of note, the cytokine IL-2 has the opposite effect to IL-15 and can inhibit proliferation by the TCE, a phenomenon that has also been observed with normal memory $CD8^+$ T cells [49, 60]. *In vitro* culture of Sendai-specific TCE yields similar results, where TCE proliferation is impaired in the absence of IL-15 [23]. Interestingly, IL-7 does not have a direct effect on TCE proliferation, suggesting that IL-7 is not critical for cell division, albeit it has not been determined whether this cytokine is important for enhanced survival of TCE [49].

These studies support a role for IL-15 in the proliferation of TCE; however, it is not clear whether this cytokine is responsible for the increased rate of turn over by TCE. The role of IL-15 in the maintenance of memory T cell populations has been difficult to interpret, as tools to identify the IL-15R α subunit by flow cytometry have not been available. Thus, studies that have aimed to elucidate a role for IL-15 on TCE proliferation have focused on IL2Rβ, the receptor subunit shared between IL-2 and IL-15. These studies demonstrate that TCE have a modest increase in the expression of IL2Rβ on their surface compared to non-TCE [50] and blocking IL2Rβ signaling decreases proliferation by TCE [49]. However, it is not known how the TCE may respond when IL-15 signaling alone is blocked, or whether there is a difference in the density of IL-15 receptor expression on the cell surface, which may provide clues to the cells sensitivity to IL-15. In summary, it is possible that TCE occur as a result of dysregulated homeostatic proliferation, or a failure to adapt to environmental alterations that are associated with age.

HYPOTHESIS: THE IMMUNE SYSTEM BEGINS ITS DECLINE EARLY IN LIFE

Age has been strongly associated with the appearance of TCE, which begs the question: does the aged environment drive the development of clonal expansions? One of the most striking changes that occur with age is the significant reduction in thymic output of naïve T cells, resulting from involution of the thymus that is initiated during adolescence [8], leading to a decline in the naïve T cell repertoire [13]. Although the appearance of TCE correlates with the marked reduction of the naïve T cell compartment, and is accelerated in thymectomized mice [53], there is no direct evidence to support that these age-associated changes impact homeostasis of the memory T cell repertoire and initiate the development of TCE.

Alternatively, we believe these large outgrowths that arise from the pre-existing memory T cell pool, develop by mechanisms independent of the aged-associated reduction in diversity of the naïve T cell repertoire. It is possible the TCE commonly analyzed represent the extreme end of a broader spectrum of dysregulation that only becomes apparent over time. In this case, we may be missing smaller perturbations in younger mice that aren't detected by the current techniques used to identify TCE. For example, virus-specific TCE that develop from the memory T cell pool following acute respiratory virus infection are typically identified when the antigen-specific $CD8^+$ T cells occupy greater than 20% of the entire $CD8^+$ T cell pool [23]. However, it is likely that a single $CD8^+$ T cell clone first begins to dominate the antigen-specific $CD8^+$ T cell population, which initially would not increase the size of the total antigen-specific T cell compartment. We hypothesize that the memory T cell pool generated following acute respiratory virus infections becomes perturbed very early, resulting in the generation of dominant T cell clones that possess a competitive advantage and gradually overtakes the $CD8^+$ T cell pool, culminating in a large TCE that thrives in the aged environment (Fig. **3**).

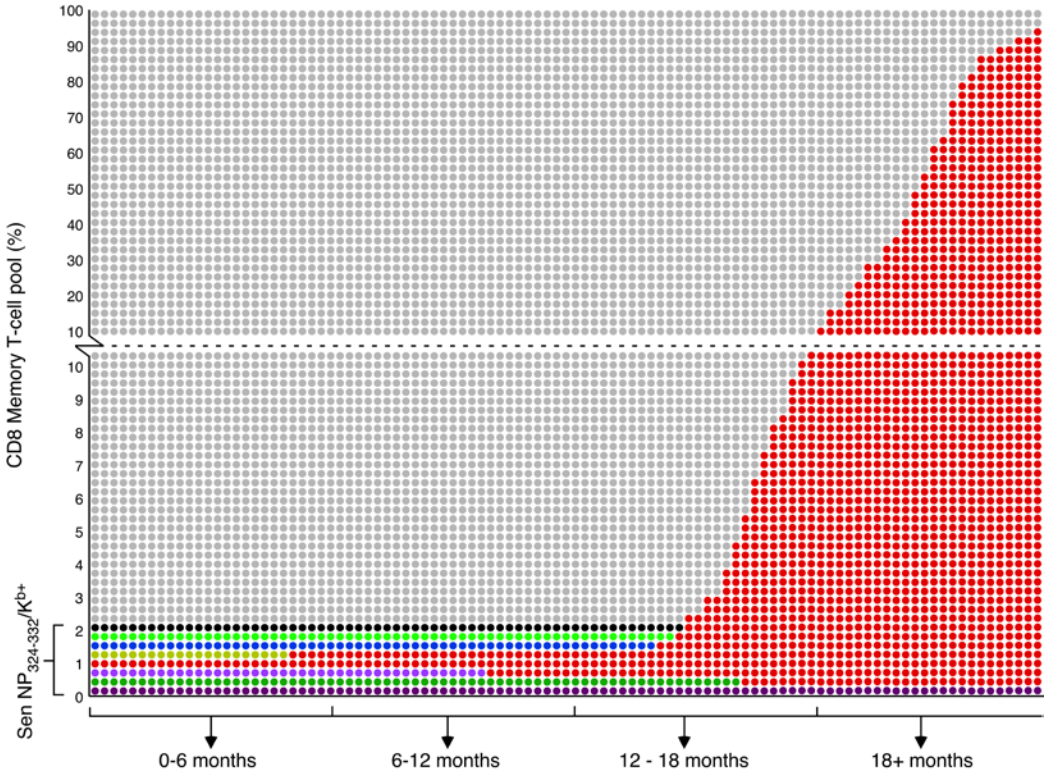

Fig. (3). Smaller perturbations occur within the memory T cell pool in young mice.
Schematic representing individual clones that comprise the memory T cell response over time. Grey symbols indicate non Sendai-specific $CD8^+$ memory T cells, colored symbols indicate individual clones within the Sendai-specific $CD8^+$ T cell pool. Between 0-12 months the frequency of Sendai-specific $CD8^+$ T cells represents approximately 2% of the total memory T cell pool, however the frequency that individual clones occupy within the Sendai-specific $CD8^+$ T cell pool changes as early as 8 months (red clone). Over time a single Sendai-specific $CD8^+$ T cell clone begins to dominate the antigen-specific T cell pool, without altering the total frequency. As a result of continued expansion, the TCE manifests in advanced age (>18 months), representing >95% of the total memory T cell pool.

Determining when TCE first begin to develop will be critical for understanding the mechanisms involved in their generation. If age is not a driving factor, are environmental cues important, or are TCE a consequence of an intrinsic defect? It is possible that homeostatic regulation in some clones is uncoupled from environmental signals and their proliferation goes unchecked. These questions can be addressed provided we gain a better understanding of the kinetics of TCE development.

CONCLUDING REMARKS

In this review we have discussed evidence clearly demonstrating that the diversity of the $CD8^+$ T cell repertoire progressively declines with age. Maintaining the naïve and memory T cell repertoire is vital for mounting robust immune responses against new and reemerging pathogens. In addition, the diminishing $CD8^+$ T cell repertoire may also lead to undesirable immune responses, such as those that are mediated by cross-reactive $CD8^+$ T cells. Importantly we believe that the diminishing diversity of the naïve and memory T cell repertoire are initiated *via* separate mechanisms. The maintenance of the naïve T cell pool is dependent on export of new T cells from the thymus, which is significantly diminished in the aged leading to holes in the $CD8^+$ T cell repertoire. The diversity of the memory T cell pool, however, is increasingly compromised by the development of TCE. We predict that the initiation of TCE is not solely dependent on age, but rather a consequence of the normal long-term maintenance of the memory T cell pool.

These observations highlight significant differences between homeostasis of long-term memory and aged naïve T cells. Gaining a better understanding of how the naïve and memory T cell compartments are maintained over time will significantly aid in the development of more effective vaccines that overcome the increasingly dysregulated immune response in the elderly.

CONFLICT OF INTEREST

None declared.

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